EZMeta™ Acid Soil Available Phosphorous Colorimetric Assay Kit

Cat #: D-AKC4014

Size: 96T

Storage: Stored at 4°C for 12 months, protected from light

Product Information

Applicable samples: Acidic Soil

Assay Principle

Available phosphorus is a phosphorus component that can be absorbed by plants in soil, including all water-soluble phosphorus, part of adsorbed phosphorus and organic phosphorus. Available phosphorus in soil is one of the main factors limiting plant growth. Available phosphorus were extracted by double acid method, determined by molybdenum antimony anti colorimetry. The material formed by molybdenum blue and phosphate had a characteristic absorption peak at 660 nm. The phosphorus content was calculated by measuring the absorbance at 660 nm.

Materials Supplied and Storage Conditions

Kit components	Size （96 T）	Storage conditions
Extraction Buffer	100 mL	4°C
Reagent I	6.6 mL	4°C
Reagent II	1	4°C, protected from light
Reagent III	1×3	4°C, protected from light
Standard	1 mL	4°C

Materials Required but Not Supplied

·Microplate reader or visible spectrophotometer capable of measuring absorbance at 660 nm

·96-well plate or microglass cuvette, precision pipettes, disposable pipette tips

·Thermostatic shaker, centrifuge, 30-50 mesh sieve

·Deionized water

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

Reagent I: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

Reagent II: Prepare before use, add 2 mL deionized water and fully dissolve before use. Store at 4°C, protected from light.

Reagent III: Prepare before use, add 1 mL deionized water to each tube according to the dosage, and use after fully dissolving. Store at 4°C, protected from light.

Working Reagent (50 samples can be measured): Prepare before use; Take one EP tube and add 660 µL Reagent I, and 100 µL Reagent II, and then add 240 µL Reagent III, fully mixed for use; According to the number of samples, the volume of each reagent can be reduced proportionally.

Note: Working Reagent should be prepared before and must be used as soon as possible use. It's normal color is light yellow. If it appeared blue or other colors, it will be invalid.

Standard: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

Standard preparation: Use 1 µmol/mL standard, prepare standard curve dilution as described in the table.

Num.	Standard Volume	Extraction Buffer Volume (µL)	Concentration (µmol/mL)
Std.1	50 µL of 100 µL 1 µmol/mL	150	0.25
Std.2	100 µL of Std.1 (0.25 µmol/mL)	100	0.125
Std.3	100 µL of Std.2 (0.125 µmol/mL)	100	0.063
Std.4	100 µL of Std.3 (0.063 µmol/mL)	100	0.031
Std.5	100 µL of Std.4 (0.031 µmol/mL)	100	0.016
Std.6	100 µL of Std.5 (0.016 µmol/mL)	100	0.008
Blank	0	100	0

Sample Preparation

Note: Fresh samples are recommended. If the experiment is not carried out immediately, the samples can be stored at -80°C for several weeks. During the determination, the temperature and time of thawing should be controlled. When thawing at room temperature, the sample should be thawed within 4 h.

The fresh soil sample was air dried, sieved through 30-50 mesh sieve, weighed about 0.1 g of soil sample, added with 1 mL Extraction Buffer, extracted with shaking for 1 h at 37°C, centrifuged at 10,000 g for 10 min at 25°C, and the supernatant was taken for test.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 660 nm, visible spectrophotometer was returned to zero with deionized water.

2. Assay procedure (The following were operated in the 96-well plate or microglass cuvette):

Reagent	Blank Well (μL)	Standard Well (μL)	Test Well (μL)
Supernatant	0	0	40
Extraction Buffer	40	0	0
Standard	0	40	0
Working Reagent	20	20	20
Deionized Water	140	140	140

3. After mixing, keep at 25°C for 30 min, and measure the absorbance at 660 nm. The absorbance of blank well, standard well, test well recorded as A_{Blank}, $A_{Standard}$ and A_{Test}. Finally, calculate $\Delta A_{Test}=A_{Test}-A_{Blank}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note: Blank well and standard well only need to measure 1 time. In order to guarantee the accuracy of experimental results, pre-experiments are suggested use 2-3 samples with potential significant difference. Increase the sample quantity appropriately if the value of ΔA_{Test} is too low, Once ΔA_{Test} is greater than 1.0, the sample can be appropriately diluted with Extraction Buffer, the calculated result multiplied by the dilution factor, or decrease the sample quantity appropriately.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Drawing of standard curve:

With the concentration of the standard solution as the y-axis and the $\Delta A_{Standard}$ as the x-axis, draw the standard curve, get the standard equation y=kx+b, and bring the ΔA_{Test} into the equation to get the x value (μmol/mL).

2. Calculation of available phosphorus content:

Available phosphorus content (μmol/g soil sample)=$x \times V_{Sample} \div (V_{Sample} \times W \div V_{Total\ sample})$**=10×x**

V_{Sample}: added sample volume, 0.04 mL; $V_{Total\ sample}$: added Extraction Buffer volume, 1 mL; W: sample weight, 0.1 g.

Typical Data

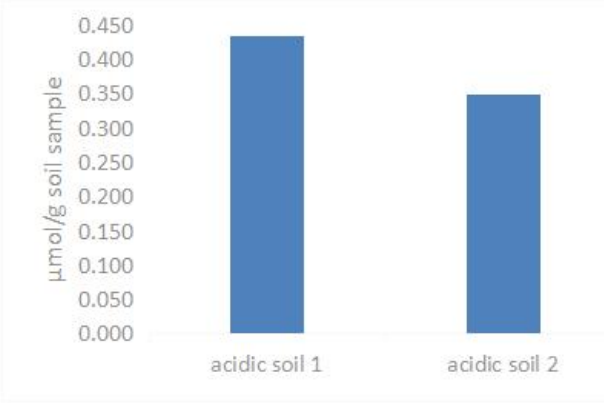

Fig.1. Determination available phosphorus in acidic soils by this assay kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.